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Abstract: The classification of human tumors based on molecular criteria offers tremendous clinical potential; however, discerning critical and "druggable" effectors on a large scale will also require robust experimental models reflective of tumor genomic diversity. Here, we describe a comprehensive genomic analysis of 101 melanoma short-term cultures and cell lines. Using an analytic approach designed to enrich for putative "driver" events, we show that cultured melanoma cells encompass the spectrum of significant genomic alterations present in primary tumors. When annotated according to these lesions, melanomas cluster into subgroups suggestive of distinct oncogenic mechanisms. Integrating gene expression data suggests novel candidate effector genes linked to recurrent copy gains and losses, including both phosphatase and tensin homologue (PTEN)-dependent and PTEN-independent tumor suppressor mechanisms associated with chromosome 10 deletions. Finally, sample-matched pharmacologic data show that FGFR1 mutations and extracellular signal-regulated kinase (ERK) activation may modulate sensitivity to mitogen-activated protein kinase/ERK kinase inhibitors. Genetically defined cell culture collections therefore offer a rich framework for systematic functional studies in melanoma and other tumors.

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Abstract

The classification of human tumors based on molecular criteria offers tremendous clinical potential; however, discerning critical and 'druggable' effectors on a large scale will also require robust experimental models reflective of tumor genomic diversity. Here, we describe a comprehensive genomic analysis of 89 melanoma short-term cultures and cell lines. Using an analytical approach designed to enrich for putative 'driver' events, we show that cultured melanoma cells encompass the spectrum of significant genomic alterations present in primary tumors. When annotated according to these lesions, melanomas clustered into subgroups indicative of distinct oncogenic mechanisms. Integrating gene expression data suggests novel tumor-promoting mechanisms, as exemplified by chromosome 10 deletions. Finally, sample-matched pharmacologic data shows that *FGFR1* mutations and ERK activation may modulate tumor sensitivity to MEK inhibitors. Genetically-defined cell culture collections may therefore offer a rich framework for systematic functional studies in melanoma and other tumors.

Introduction

The recognition that cancer is fundamentally a genetic disease[1,2], combined with an expanding repertoire of targeted small molecules[3-8], provides grounds for optimism that genome-based therapeutics may ultimately prove broadly applicable to human cancer. However, the rational deployment of targeted anti-cancer agents is often encumbered by an inability to identify a priori those tumors whose unique biology confers heightened susceptibility to a particular treatment. Considerable insights into tumorigenesis have derived from functional studies involving cultured human cancer cells (e.g., established cell lines, short-term cultures, etc.); however, the overall relevance of tissue culture models to the complexities of *in vivo* tumor biology has often been questioned. Despite their limitations, cancer cell line collections whose genetic alterations reflect their primary tumor counterparts should nonetheless provide malleable proxies that

facilitate mechanistic dissection and therapeutic development. Indeed, matched genomic and experimental data derived from these models may refine hypothesis generation and reveal new insights, as evidenced by recent studies[9,10]. Such results may also be integrated with analogous data from clinical specimens to infer molecular subtypes and therapeutic vulnerabilities in tumors that manifest the relevant genomic changes[11-13].

When it escapes early detection, malignant melanoma usually becomes a highly lethal malignancy that is refractory to existing therapeutic avenues[14,15]. However, whereas many other solid tumors lack robust *in vitro* counterparts, melanoma offers an attractive experimental platform for systematic functional characterization of cancer genomic aberrations. Melanoma cells from patients with advanced disease proliferate readily *in vitro*; thus, hundreds of 'short-term' melanoma cultures and established cell lines have been generated[16-18]. Melanoma short-term cultures have typically undergone a relatively small number of passages outside of the patient, and the majority of these lines proliferate readily under standard laboratory conditions. In principle, then, a cultured melanoma collection that reflects the diversity of genomic aberrations observed in primary melanomas should facilitate the characterization of critical and 'druggable' effectors linked to key molecular lesions in this malignancy.

Results

The genomic diversity of melanoma *in vitro* and *in vivo*

To enhance global knowledge of melanoma genomic alterations while also establishing a robust experimental system for downstream functional characterization, we generated comprehensive genomic data for 78 short-term melanoma cultures (median = 9 passages *in vitro*; range = 5-51), together with 11 established melanoma cell lines. Chromosomal copy number and loss-of-heterozygosity (LOH) alterations were examined using high-density single-nucleotide polymorphism (SNP) arrays[9,19,20], and the mutation status of 17 known oncogenes was

interrogated by Sanger sequencing or a mass spectrometry-based mutation profiling approach described previously[21]. Gene expression patterns were determined for 88 cultured melanoma lines and 5 normal melanocyte lines using a high-throughput microarray platform[22] (Affymetrix, Inc.; see Methods). Altogether, 118 cultured melanoma lines were examined by one or more of these platforms.

Since tumor chromosomal alterations are often non-focal, we next wished to predict which genetic changes represented statistically significant events (presumably enriched for "driver" alterations) in melanoma. To accomplish this, we employed an algorithm termed Genomic Identification of Significant Targets in Cancer (GISTIC; Beroukhim *et al.,* submitted). This method systematically computes a significance score at each locus across the genome using smoothed microarray/CGH copy number data, based on the frequency and amplitude of each alteration (G score; see Methods). After permutation testing and multiple hypothesis correction, resulting false discovery rate (FDR)-corrected p values are plotted as a function of chromosomal location. Significant genomic regions of gain or loss are denoted by peaks in the GISTIC plot; the underlying chromosomal regions may then be inspected for candidate target genes.

The GISTIC output revealed thirteen major regions of significant copy number gain and fourteen regions of significant copy loss in the cultured melanoma collection (Figs. 1a and 1b; Tables 1 and 2). The most significant region of copy gain was located on chromosome 7q (Fig. 1a). This locus harbors the BRAF oncogene, which contains activating point mutations in >50% of cutaneous melanomas[23] and in 60% of samples examined here. This suggests that mutated BRAF is also frequently amplified in melanoma. Additional prominent GISTIC copy gain peaks localized exquisitely to *MITF*, the master transcriptional regulator of melanocyte development and an amplified oncogene in 10-15% of melanomas[9]; and to *CCND1*, a well-known oncogenic cell cycle regulator (Fig. 1a). The most prominent region of copy loss spanned CDKN2A, an

established melanoma tumor suppressor gene[24,25] (Fig. 1b). Aside from these expected findings, the majority of GISTIC peaks pinpointed genomic regions implicated previously in melanoma[26-28] (Supplementary Fig. 1), but whose target genes remain largely unknown (Tables 1 and 2). Integration of sample-matched gene expression data enabled identification of candidate effector genes in several instances (Tables 1 and 2). Thus, GISTIC analysis afforded a robust and unbiased means to identify key genomic loci whose target genes may contribute importantly to melanoma biology.

Theoretically, cultured human cancer cell collections may represent a biased malignant subset that fail to encompass the pathophysiology relevant in primary tumors. To address this at a genomic level in cutaneous melanoma, we performed GISTIC analysis on bacterial artificial chromosome (BAC) array CGH data derived from 70 primary cutaneous melanomas (Curtin ref; see Methods) and compared the resulting significance values to those observed in primary tumors. As shown in Figs. 1a and 1b, the landscape of genomic alterations was strikingly similar between cultured cells and primary tumors, even with respect to subtle contours within the GISTIC plots. Some significant alterations present in cultured cells were absent in primary tumors (e.g., gains on chromosome 3p14 (MITF) and chromosome 15q, and a region of loss on 4q); in most cases, these were attributable to low BAC clone coverage in the primary tumor data. Conversely, a single GISTIC peak centered on chromosome 13q33 exhibited reduced significance in cultured cells compared to primary tumors. Interestingly, the underlying 3.2 Mb locus spanned the *ERCC5* excision repair gene, and this deletion showed marked enrichment in primary melanomas from chronic sun-damaged (CSD) skin[26] (Supplementary Figure 2a, 2b, 2c). These findings raised the possibility that deletions involving *ERCC5* may contribute to melanoma genesis in the setting of chronic sun damage.

To confirm the similarities between cultured melanoma cells and primary melanomas, we performed pair-wise Pearson correlations between GISTIC plots of cultured melanoma data, primary melanomas, and a series of additional solid tumors whose chromosomal aberrations had been examined previously (Supplementary Table 1). Notably, GISTIC correlations between cultured melanoma cells and primary cutaneous melanomas from non-CSD skin were stronger than any pair-wise coefficient observed among all other primary tumor sets, thereby providing a rigorous quantitative metric of genomic similarity. These data suggested that, in aggregate, cultured melanoma collections might provide a genetically appropriate model system for systematic functional genomic characterization.

Genome-wide measurements of loss-of-heterozygosity (LOH) in melanoma

High-density SNP arrays also enable inference of LOH[29], even in the absence of matched normal DNA[30]. To identify significant LOH regions within our cultured melanoma collection, we predicted LOH patterns within each sample using a hidden Markov model[30], and applied the GISTIC algorithm to the resulting LOH segments (see Methods). A strong concordance was observed between regions of LOH and copy loss (Fig. 1c), suggesting that most melanoma LOH events occur by hemizygous deletion. The major exceptions to this pattern occurred at chromosomes 5q and 17p, where LOH in the absence of significant hemizygous deletion ("copy-neutral" LOH) was apparent. Although the relevant tumor suppressor at chromosome 5q has not been identified, the LOH peak at chromosome 17p centers on the well-known *TP53* tumor suppressor gene (Fig 1c). This suggests that copy-neutral LOH may represent an important mechanism of p53 inactivation in melanoma. In a subset of short-term culture samples, a segment of chromosome 4q appeared to contain significant hemizygous deletion in the absence of LOH (Fig. 1c). This unusual phenomenon, which could occur by whole genome duplication followed by copy loss and biallelic retention, implies that this region of the melanoma genome might be relatively refractory to LOH or uniparental disomy.

Molecular classification of melanoma based on chromosomal aberrations

We next sought to determine if significant chromosomal events might organize the melanoma samples into molecularly distinct and/or biologically relevant subsets. To address this, we applied established unsupervised learning methods[31] to the lesions identified by GISTIC (see Methods). As shown in Figure 2a, hierarchical clustering grouped GISTIC-annotated cultured melanoma samples into four main branches. The major subcluster (n = 33 samples; Fig. 2a, cluster #3) was enriched for several prevalent alterations, most notably gains on chromosome 7p/7q and losses on 9p21, 4q34, and 10p/10q. This subcluster was associated with a marked enrichment in BRAF mutation compared to NRAS mutation in both the cultured and primary melanoma sets (Fig. 2a), thereby highlighting the observation that mutated BRAF is commonly amplified, at least to low levels, in melanoma (Supplementary Fig. 3c, 3d, 3e).

A second major subcluster (n = 24 samples; Fig. 2a, cluster #4) was characterized by essentially the same genomic alterations, except for the notable absence of chromosome 10 deletions. NRAS mutations exhibited a higher prevalence in this subcluster than in cluster #3 (Fig. 2a). Nonetheless, this cluster was also characterized by a high frequency of 7q gain, even in the setting of NRAS mutation, suggesting that BRAF-independent effectors located on chromosome 7q may also contribute to melanoma biology. A third group (n=20 samples) was characterized by a relative paucity of copy number alterations (“sparse”; Fig. 2a, cluster #1). Finally, a relatively small branch (n = 13 samples, cluster #2) was moderately enriched for gains at 6p, 17q and 7p, and losses on 6q. Overall, these results suggested that GISTIC lesions may promote biologically relevant molecular groupings of melanoma tumor samples.

Predicting genes targeted by chromosome 10 deletions in melanoma

In melanoma, as in many solid tumors, most statistically significant chromosomal aberrations span large genomic regions whose target genes are unknown[26-28]. Among many other lesions, this pattern is exemplified by the gains and losses involving chromosomes 7 and 10, respectively—two prominent drivers of the GISTIC-based melanoma subclusters described above. Although the most significantly "amplified" region on chromosome 7 centers at 7q34 (contains *BRAF*; Fig. 1a), most chromosome 10 copy losses cover the entire chromosome, punctuated by a more focal GISTIC peak at 10q23.31 (Fig. 1b and Supplementary Figure 1a and 1b) spanning the *PTEN* tumor suppressor gene. To a first approximation, these observations are consistent with a model in which chromosome 7 gains and 10 deletions enhance oncogenic BRAF and diminish PTEN expression, thereby suggesting cooperating effects of these two cancer genes in melanoma, as suggested previously[32]. On the other hand, whereas chromosome 10 deletions are highly prevalent even in early-stage melanomas[26] (Figure 1b), the relative contribution of PTEN inactivation in melanoma establishment remains unclear.

To investigate this, we examined whether pan-chromosome 10 deletions were associated with functional PTEN inactivation, either through genetic mutation or mRNA/protein loss. As shown in Figure 3a, only five of 89 short-term cultures and cell lines with chromosome 10 deletions also contained focal PTEN homozygous deletions; similar results were observed in an independent melanoma cell line panel described previously (Supplementary Figure 3a). Consistent with prior DNA sequencing analyses of short-term melanoma cultures and primary tumors[33,34], only 5 of 98 lines examined here contained inactivating *PTEN* mutations; and only two of these samples also harbored hemizygous loss or LOH of chromosome 10. Using sample-matched gene expression data, we then examined PTEN mRNA expression in relation to genomic deletion. Here, whereas PTEN homozygous deletion correlated with a marked reduction in PTEN expression, <50% of samples with hemizygous deletions exhibited

decreased PTEN levels when compared to samples without alterations at this locus (Fig. 3B). Similar results were observed following immunoblot analysis of PTEN protein levels in a subset of samples (Fig. 3c). These observations raised the possibility that an as-yet uncharacterized tumor suppressor gene(s) located on chromosome 10 might promote tumor establishment in a large fraction of melanomas.

To identify additional candidate tumor suppressor genes enacted by non-focal chromosome 10 deletions in a non-biased manner, we partitioned our samples into tertiles based on the ranked magnitude of chromosome 10 loss (see Methods) and segregated the two extreme tertiles according to the class distinction 'chromosome 10-deleted' (20 samples) versus 'chromosome 10-wild-type' (21 samples). We then performed a genome-wide significance analysis (SAM) using sample-matched gene expression data. As shown in Fig. 3d, 43 unique transcripts exhibited significantly diminished expression in association with chromosome 10 deletion at a delta value of 1.344 (median false number of genes = 0). Interestingly, two chromosome 10 genes with putative tumor suppressor roles—*CUL2* (chromosome 10p11) and *KLF6* (chromosome 10p14)—scored in this analysis, whereas *PTEN* failed to reach statistical significance (Figure 3D). *CUL2* encodes a cullin protein whose yeast homologues negatively regulate the cell cycle[35]; CUL2 also forms an E3 ubiquitin ligase complex with the VHL tumor suppressor protein[36,37]. *KLF6* encodes a zinc-finger transcription factor implicated as a tumor suppressor in several malignancies[38-40]. Thus, *CUL2* and *KLF6* represent intriguing candidate melanoma tumor suppressor genes targeted by chromosome 10 deletions.

Modifiers of RAF/MEK dependency in melanoma

The establishment of an experimentally tractable system reflective of melanoma genomic diversity provides a means to identify molecular predictors and modifiers of therapeutic response in this highly chemo-resistant malignancy. Since most cutaneous melanomas harbor

activating point mutations in BRAF or NRAS[23,41] (two key effectors of the MAP kinase signaling cascade), several ongoing clinical trials are investigating the efficacy of RAF and MEK inhibitors in this setting. To identify genetic/molecular modifiers of MAP kinase dependency that might influence therapeutic response, we examined cellular response to pharmacologic MAP kinase inhibition in a panel of 31 short-term cultures using the MEK inhibitor CI-1040. $BRAF^{V600E}$ mutation was invariably associated with sensitivity to MEK inhibition as shown previously[10] (e.g., sub-micromolar CI-1040 GI50 values; Figure 4A); although one $BRAF^{V600E}$ line (WM853-2) was moderately less sensitive to CI-1040. In contrast, NRAS-mutant melanomas exhibited highly variable MAP kinase dependencies; whereas 4 NRAS-mutant lines with codon 61 mutations showed CI-1040 sensitivity patterns similar to the $BRAF^{V600E}$ panel, two lines harboring codon 12/13 NRAS mutations were 'indifferent' to CI-1040 treatment. PTEN protein loss was more common in lines showing decreased sensitivity to CI-1040 (Figure 4a). Surprisingly, a short-term culture harboring the $BRAF^{K601E}$ mutation also showed insensitivity to CI-1040. Together, these data suggested the presence of additional genetic or molecular modifiers of melanoma MAP kinase dependency apart from BRAF or NRAS mutation.

To investigate this in more detail, we examined the associated genomic and protein data in the melanoma lines described above. Despite the diversity of CI-1040 GI_{50} values, a similar degree of target inhibition was evident in several representative lines, as measured by p-ERK immunoblotting studies (Supplementary Figure 4a). A genome-wide analysis of correlated chromosomal alterations and expressed genes, summarized in Supplementary Figure 5, was encumbered by the relatively small CI-1040-insensitive sample size (3 samples). However, two lines harboring the $FGFR1^{S125L}$ mutation exhibited markedly enhanced sensitivity to MEK inhibition when compared to the A375 control cell line (Fig. 4b). In contrast, 'steady-state' immunoblot analyses of MEK and ERK found that p-ERK levels were markedly elevated in all CI-1040-insensitive lines, with concomitant downregulation of total MEK protein in the three

most resistant lines (Figure 4c). These data suggest the intriguing possibility that novel signaling or feedback regulatory mechanisms may affect MAP kinase activation/dependency in melanoma, and that concomitant measurement of oncogene mutations and p-ERK levels may refine knowledge of melanoma patients likely to respond to targeted RAF or MEK inhibition.

Discussion

The unprecedented opportunities of cancer genomics also present new challenges to the identification of 'target-able' tumor mechanisms. In particular, characterizing the downstream effectors of common but often non-focal genomic alterations requires tractable *in vitro* models reflective of these genetic events. Our global genomic analyses indicate that melanoma short-term cultures appear to encompass the spectrum of essential genomic diversity present in primary cutaneous melanomas, suggesting that these may offer a robust platform for functional genomic characterization. Moreover, the high-resolution statistical delineations enabled by the GISTIC analytical framework strongly suggests (but does not prove) that such regions contain genes whose perturbation contributes causally to melanoma genesis or maintenance.

These analytical refinements also call renewed attention to instances where a full chromosome or chromosome arm displays a common tumor-associated alteration. In melanoma, deletions and LOH involving chromosome 10 provide an instructive example. Several previous studies have implicated inactivation of the PTEN tumor suppressor gene as a key (epi)genetic event in melanoma. While our results support the notion that PTEN loss provides a driver alteration in some melanomas, they also suggest an alternative model wherein chromosome 10 losses provide a polygenic or otherwise PTEN-independent melanoma genesis mechanism(s). Overall, the tendency towards large chromosomal perturbations in cancer underscores the future need for systematic functional studies in genetically-characterized tumor model systems.

Application of hierarchical clustering to a matrix of GISTIC lesions yields melanoma subclasses whose characteristics are suggestive of mechanistic relevance. When applied broadly in cancer genomics, these analyses may help reduce the complexity of cancer genome aberrations while also laying a robust groundwork for molecular classification and downstream functional approaches. In the future, genetically annotated melanoma lines could be subjected to systematic RNAi studies informed by GISTIC or related statistical information. Genes located within statistically credentialed genomic regions whose knockdown modulates a cancer phenotype of interest may illuminate 'druggable' cellular pathways linked to tumor genomic events identifiable *in situ*.

Finally, cell culture models reflective of *in vivo* tumor genetic diversity offer an attractive avenue to identify molecular features that modify the efficacy of therapeutic agents. In melanoma, the MAP kinase pathway is commonly activated by BRAF or NRAS oncogene point mutations, and BRAF(V600E) mutation is associated with sensitivity to RAF or MEK inhibition. Our data suggest that in some contexts, NRAS or FGFR1 mutations may also confer sensitivity to MAP kinase pathway inhibition, but that high p-ERK may correlate with insensitivity to these inhibitors. While these studies require functional validation, they demonstrate how cultured cell models facilitate orthogonal analyses of genomic and protein data to yield new insights into targeted therapeutic response. Overall, the large-scale application of these approaches to genetically annotated cancer cell culture models should provide a rich framework for extraction of key dependencies from tumor genomic data, thereby offering new therapeutic possibilities in melanoma and many other solid tumors.

Materials and Methods:

Cell culture and reagents.

Short-term melanoma cultures were selected from cryopreserved collections at the Wistar Institute (62 lines), the University Hospital of Zurich (24 lines), and the Dana-Farber Cancer Institute (32 lines). Established melanoma cell lines were provided by Dr. Susan Holbeck (National Cancer Institute) and Dr. David Fisher (Dana-Farber Cancer Institute). All cell lines and short-term cultures were cultured in RPMI medium (MediaTech) supplemented with 10% fetal bovine serum (MediaTech), except for the Dana-Farber short-term cultures, which were grown in Dulbecco's modified Eagle medium (MediaTech) supplemented with 10% serum. For some Wistar lines, tissue culture dishes were first coated with 1% porcine gel solution (Sigma #G1890-100G) to enhance cell adherence.

High-density SNP array hybridization.

Genomic DNA was prepared from near-confluent cells using the DNEasy Tissue Kit (Qiagen) according to the manufacturer's instructions. For highly pigmented cultures, 1:1 phenol-choloroform extractions followed by centrifugation were performed prior to column purification. SNP array data was generated using either the *Sty*I chip from the 500K Human Mapping Array set (Affymetrix, Inc.) (58 samples) or the *Xba*I chip from the 50K Human Mapping Array set (31 samples). Array experiments using 250 ng of genomic DNA were carried out in 96-well format using a Biomek FX robot with dual 96 and span-8 heads (Beckman Coulter) and a GeneChip Fluidics Station FS450 (Affymetrix, Inc.), as described in **Supplementary Methods**. After hybridization, microarrays were scanned using the GeneChip Scanner 3000 7G (Affymetrix, Inc.) to generate .cel and .txt files. Subsequent data processing steps are detailed in **Supplementary Methods.**

High-throughput oncogene mutation profiling and DNA sequencing.

Genomic DNA was genotyped for 238 known mutations in 17 oncogenes as described previously.[21] Alternatively, BRAF and NRAS mutations were determined by Sanger sequencing (Agencourt). All samples were sequenced for *PTEN* coding and splice junction mutations using pre-validated exon primers (Agencourt) and analyzed with Mutation Surveyor (SoftGenetics, LLC.).

Gene expression profiling.

RNA was purified by a TRIzol extraction protocol (Invitrogen), as described in **Supplementary Methods**. Affymetrix HT-HGU133A chips were used for generation of gene expression data.[22] With 2.5 µg of RNA, synthesis of the cRNA target product, hybridization to the microarray, and scanning of the arrays followed Affymetrix manufacturer's protocols. Reagents and products associated with the GeneChip assay were used in 96-well plate format and the plate was scanned with the Affymetrix HT scanner. Scanned data was converted to average difference values using MAS 5.0 (Affymetrix), as described in **Supplementary Methods**.

BAC array data processing.

Previously reported BAC array CGH primary melanoma data[26] was downloaded from the GEO database (http://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE2631). The genomic markers were converted from hg16 to hg17 using the UCSC genome browser (http://genome.ucsc.edu/cgi-bin/hgLiftOver). Log_2-ratio values were smoothed using the GLAD segmentation algorithm. BAC markers in regions of known copy number polymorphisms were removed (http://projects.tcag.ca/variation/) prior to downstream analysis.

Genomic Identification of Somatic Targets in Cancer (GISTIC) analysis.

Details of the GISTIC algorithm are described elsewhere (Beroukhim *et al.*, submitted). Briefly, each SNP array or CGH data set is defined in terms of copy-number values for *N* markers along

the genome in *M* tumor samples. To identify significant regions of amplification and deletion, the GISTIC algorithm considers both the frequency *F* of an amplification or deletion above (or below) a certain threshold, and the amplitude of that alteration *A* beyond a certain threshold defined by normal samples. Thereby, a genome significance score (G score) $G = F \times A$ is defined over *N* markers for all *M* samples. At each marker *n* the computed metric *G* is compared to the null hypothesis – a background metric defined by permutation testing across all the markers. Correcting for multiple hypotheses, we generate a false-discovery rate (q-value) at each marker *n,* aggregating information from *M* samples. Using segmented copy number data from each sample, the minimal common region of overlap is used to define boundaries of peaks in the distribution of q-values. A "wide peak" boundary is determined by samples remaining after leaving out the individual samples that define the borders of the "narrow peak." This wide peak reduces the influence of individual samples in dictating peak borders.

Inference of Loss-of-heterozygosity.

A Hidden Markov Model from dChipSNP software (August 16, 2006 build) that considers haplotype information was used to infer LOH calls as described previously[30] (**Supplementary Methods**).

Hierarchical clustering using genomic alterations.

An input binary matrix generated from the GISTIC algorithm (see above). For each lesion defined by GISTIC, a "1" was assigned to a lesion *i* in a given sample *j* if the middle marker in the lesion *i* region exceeded the designated $\log_2$ threshold of 0.3 for amplifications, or -0.3 for deletions. Otherwise, that cell *ij* was assigned a "0." Hierarchical clustering[42] with Euclidean distance and complete linkage was applied to this matrix using the GenePattern software package.[43]

Significance Analysis of Microarrays (SAM).

SAM was performed using TIGR MeV 4.0 (http://www.tm4.org/mev.html) to identify differentially expressed genes associated with chromosome 10 copy number loss. Samples were first grouped into 3 classes based on mean log 2 copy number over chromosome 10 (>-0.16, -0.16> >-0.44, and <-0.44). Thresholds were chosen based on a 33 and 67 percentile of copy number distribution. Calculations were done with K-nearest neighbors imputer, 10 neighbors, 100 permutations and the Tusher method[44] to select S0.

Pharmacologic growth inhibition assays.

Cultured melanoma cells were added to 96-well plates at a concentration of 1,000 (A375 and MCF7) and 3,000 (all short-term melanoma cultures and the MALME 3M cell line) cells per well. Cells were allowed to adhere overnight, and media containing serial dilutions of CI-1040 (from 100μM to $1x10^{-6}$ μM) was added. Cells were incubated for 96 hours, at which time cell viability was measured using the CellTiter-Glo viability assay (Promega). Viability was calculated as a percentage of untreated control values after background subtraction.

Western blotting and biochemical studies.

After cell lysis (**Supplementary Methods**), Western blot analysis was performed using primary antibodies against p-Erk (anti-phospho-p44/42), total ERK (anti-p44/42), p-MEK (anti-phospho-MEK1/2 (Ser217/221)), total MEK (anti-MEK1/2) (Cell Signaling Technology), Cyclin D1 (sc-20044, Santa Cruz Biotechnology), and alpha-tubulin (anti-alpha-tubulin, Cell Signaling Technology). All primary antibodies were used at a 1:1000 dilution. The secondary antibody was anti-rabbit IgG, HRP-linked (Cell Signaling Technology) at a 1:1000 dilution. Biochemical MEK inhibition by CI-1040 was examined by treating subconfluent melanoma cells with varying dilutions of CI-1040 for 24 hours. After harvest and protein preparation, Western blotting with performed with the p-ERK and total ERK antibodies described above.

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Figure Legends

Figure 1: Significant copy number and LOH alterations in melanoma

Statistically significant genomic amplifications (a) and deletions (b) pinpointed by GISTIC analysis of 89 cultured melanoma lines (dark red and dark blue, respectively) and 70 primary cutaneous melanomas (orange and light blue, respectively) are shown. Top axis: FDR-

corrected q-values (threshold FDR=0.25; green line), left axis: chromosome, bottom axis: G-score (see text). (c) GISTIC plot of inferred loss-of-heterozygosity (gray) is superimposed onto the chromosome deletion plot from (b) (dark blue) in cultured melanoma lines. Selected known melanoma oncogenes are indicated.

Figure 2. Clustering analysis of significant melanoma genomic alterations

Hierarchical clustering of GISTIC lesions (discretized smooth copy number; see Methods) by the Euclidean distance metric and complete linkage is shown. Rows: genomic lesions identified by GISTIC algorithm (see text); columns: samples. Red = presence and blue = absence of lesions denoted by cytobands (A = amplification; D = deletion; right). Mutation status of BRAF and NRAS are noted above the matrix. Major clusters are indicated by boxes labeled 1-4.

Figure 3: Integrative analysis of chromosome 10 deletions in melanoma

(a) Heatmap view of smoothed 250K SNP array data spanning the PTEN locus (red = copy gains; blue = copy losses. Samples (columns) are sorted based on copy number values derived from segmented data. (b) *PTEN* gene expression values stratified according to homozygous deletion, hemizygous loss or retention of the underlying locus. (c) Summary of PTEN protein levels in relation to chromosomal deletions affecting the *PTEN* locus. (d) Significance analysis of melanoma gene expression data. Samples are stratified according loss or retention at chromosome 10 (See Methods). The positions of CUL2, KLF6, and PTEN are shown.

Figure 4. Molecular modifiers of MAP kinase dependency in melanoma

(a) Pharmacologic GI_{50} values for the MEK inhibitor CI-1040. Mutation status for *BRAF* and *NRAS* genes, genomic status at the *PTEN* locus, and PTEN protein levels are indicated. (b) GI_{50} values for two short-term cultures harboring FGFR1 mutations are shown alongside a

control melanoma cell line (A375). (c) Western blot analyses of p-ERK, total ERK, p-MEK, total MEK and α-tubulin are shown for selected melanoma lines (CI-1040 GI_{50} values are indicated).

Table 1: Significant regions of amplification in cultured melanoma lines

Chr	Cytoband	Q-value	Narrow peak	Wide peak	Genes in Wide Region	Genes of Interest
1	1q21.3	1.48E-10	chr1:148,640,000-148,816,000	chr1:147,610,000-149,170,000	43	
3	3p13	9.36E-17	chr3:70,040,000-70,120,000	chr3:69,870,000-70,220,000	1	MITF
5	5p15.33	1.37E-01	chr5:360,000-880,000	chr5:1-1680,000	21	TERT
6	6p25.1	5.70E-08	chr6:5,040,000-5,070,000	chr6:4,070,000-5,620,000	7	
7	7q34	5.07E-26	chr7:139,030,000-140,090,000	chr7:139,030,000-140,480,000	12	BRAF
7	7p22.3	7.09E-17	chr7:2,560,000-2,760,000	chr7:2,340,000-3,100,000	7	
8	8q24.21	3.77E-04	chr8:130,710,000-131,200,000	chr8:125,960,000-131,360,000	11	MYC?
11	11q13.3	6.47E-04	chr11:68,800,000-69,060,000	chr11:68,680,000-69,320,000	6	CCND1
12	12p12.1	1.08E-01	chr12:23,210,000-23,670,000	chr12:22,710,000-23,670,000	2	
15	15q26.1	2.06E-05	chr15:88,690,000-88,890,000	chr15:87,510,000-89,110,000	26	
17	17q25.3	2.03E-07	chr17:75,860,000-76,610,000	chr17:74,750,000-77,010,000	26	
20	20q13.2	2.50E-18	chr20:49,230,000-49,880,000	chr20:49,120,000-50,620,000	4	NFATC2?
22	22q13.2	2.70E-10	chr22:39,360,000-40,170,000	chr22:39,140,000-40,220,000	15	EP300?

Table 2: Significant regions of deletion in cultured melanoma lines

Chr	Cytoband	Q-value	Narrow peak	Wide peak	Genes in Wide Region	Genes of Interest
3	3p26.1	1.21E-01	chr3:4,240,000-4,320,000	chr3:3,820,000-4,470,000	3	
4	4q34.3	9.97E-09	chr4:182,750,000-182,840,000	chr4:182,670,000-182,960,000	0	
5	5q34	1.53E-03	chr5:166,130,000-166,300,000	chr5:165,720,000-166,380,000	0	
6	6q26	2.80E-09	chr6:163,090,000-163,340,000	chr6:162,830,000-163,450,000	2	PACRG, PARK2?
8	8p23.1	2.96E-03	chr8:6,800,000-6,900,000	chr8:6,800,000-6,950,000	3	
9	9p21.3	8.50E-72	chr9:21,980,000-22,020,000	chr9:21,940,000-220,320,000	2	CDKN2A
10	10q23.31	1.10E-12	chr10:89,670,000-89,850,000	chr10:89,530,000-89,890,000	2	PTEN
10	10p15.2	1.22E-10	chr10:3,100,000-3,170,000	chr10:1,330,000-4,630,000	4	KLF6?
11	11q23.1	1.30E-07	chr11:111,920,000-111,970,000	chr11:111,820,000-112,160,000	0	
13	13q31.1	1.90E-03	chr13:84,620,000-85,090,000	chr13:84,560,000-96,340,000	16	
14	14q32.2	3.05E-02	chr14:97,930,000-98,100,000	chr14:97,610,000-98,100,000	0	
15	15q21.1	6.22E-02	chr15:42,830,000-42,920,000	chr15:42,720,000-43,420,000	13	
16	16q23.2	6.04E-02	chr16:78,590,000-78,830,000	chr16:78,410,000-78,920,000	0	
18	18q22.1	1.03E-02	chr18:62,660,000-63,040,000	chr18:62,660,000-72,570,000	24	